Progress Report on Funded Nursery Projects
Washington State Department of Agriculture

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Project Title: Developing control strategies for *Cherry raspleaf virus*

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Progress: To be submitted for all projects funded in FY05 (July 1, 2004 to June 30, 2005); and FY 06 (July 1, 2005 to June 30, 2006).

At the inception of this project, the only known sequence for *Cherry raspleaf virus* represented the smaller of two genomic RNA molecules of the flat apple disease strain. A significant report appeared this year that indicated that several lines of seed potato and mint are symptomless carriers of *Cherry raspleaf virus* in other States (Thompson, Perry & DeJong, 2004. Arch Virol 149:2141-2154). This extends both the known natural host range and the known geographical distribution of *Cherry raspleaf virus*. This raises concerns about the potential infection of apples and cherries when these crops are planted on land that has been previously used for field crops. This practice is becoming more common in the Pacific Northwest.

We have now sequenced the entire genome (that is, both RNA1 and RNA2 molecules) of the cherry raspleaf disease strains of *Cherry rasp leaf virus* from two independent sources, one in Washington and one in California. The larger RNA1 molecules are very highly conserved, whereas there is considerable variability in the smaller RNA2 molecules. The latter molecule is the one that encodes the coat proteins, and hence determines the reaction of the virus with antibodies in serological tests. In an effort to predict the reliability of ELISA, the most common serological method of virus detection, we determined the complete RNA2 sequence from additional sources. The characterization of another cherry isolate from Washington was completed, and one from Oregon is in progress. The phylogenic relationship of the viruses for which the complete sequence of RNA2 is known is presented in figure 1. The three Washington isolates are closely related, regardless of host plant. The potato isolate from Wisconsin is more distantly related, and the California cherry isolate is relatively unique when the sequence of the entire RNA2 sequence is considered:

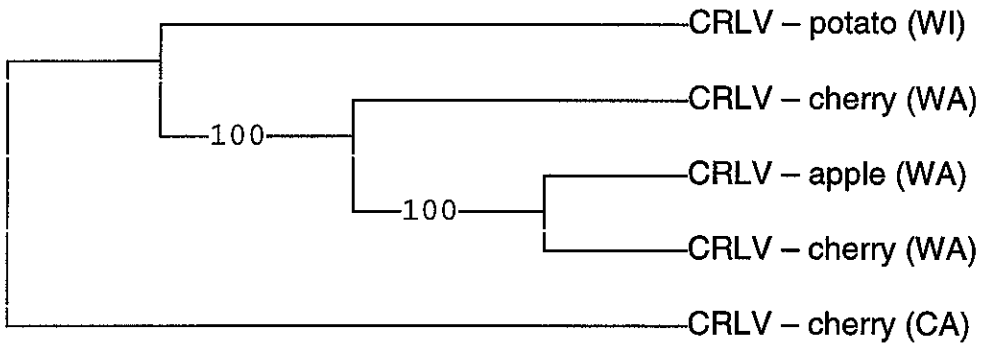

Figure 1. Phylogenic analysis of nucleotide sequences RNA2 from several CRLV isolates.

When just the predicted amino acids of the coat proteins are considered, the potato, apple and cherry isolates are between 96% to 98% identical with the exception of the California isolate from cherry that is 75% similar to the others. Nevertheless, based on this information, we predict that all of these isolates will likely respond equally to most serological reagents. We are currently expanding this analysis to include an isolate obtained from an Oregon cherry orchard.

The similarity of the predicted antigenic sites (peptide sequences that are potentially involved in antibody recognition based on models of protein structure by Kolaskar & Tongaonkar 1990 FEBS Letters 276:172-174) is illustrated in figure 2. The high degree of similarity at the potential antigenic sites suggests that one set of reagents will detect all isolates. The genes for each of the three individual coat proteins have been cloned into bacteria, their expression induced, and the proteins used to solicit the production of antibodies in rats. These trials are in their early stages, but the reaction of antibodies to the VP25 and VP20 (the first two of three fragments in the coat protein complex) looks encouraging. The antibodies produced to these proteins yields a strong reaction to the Kennewick, WA isolate of *Cherry rasp leaf virus* in crude extracts of *Chenopodium quinoa*, but reacts weakly with other *Cherry rasp leaf virus* isolates in extracts of dormant cherry wood (Table 1). This is an incomplete assessment of the antibodies, and a more thorough evaluation will be completed once more antiserum is available. It is not known at this time if the lower reading in the budwood is a reflection of low virus titer in dormant wood and the inability of ELISA to detect such low concentrations, or if the failure to detect the other isolates is the result of differences in the antigenic determinants of the virus particles. This question will be addressed.

Table 1. Preliminary evaluated of the ability of rat antibodies to detect *Cherry raspleaf virus* in crude plant extracts.

Sample	**ELISA Result (A_{405})**	
	Anti-VP25	**Anti-VP20**
Buffer blank	0.115	0.097
C. quinoa (WA)	1.938	1.408
Budwood (WA)	0.474	0.607
Budwood (WA)	0.546	0.528
Budwood (WA)	0.498	0.494
Budwood (WA)	0.617	0.831

To increase sensitivity, we are now attempting to produce the complete three peptides as a single unit. It is hoped that the proteins synthesized in this manner will more closely resemble the structure of the native virus particle, and thus solicit antibodies that react strongly with the virus as it is presented in plant extracts. This phase of the project is just beginning.

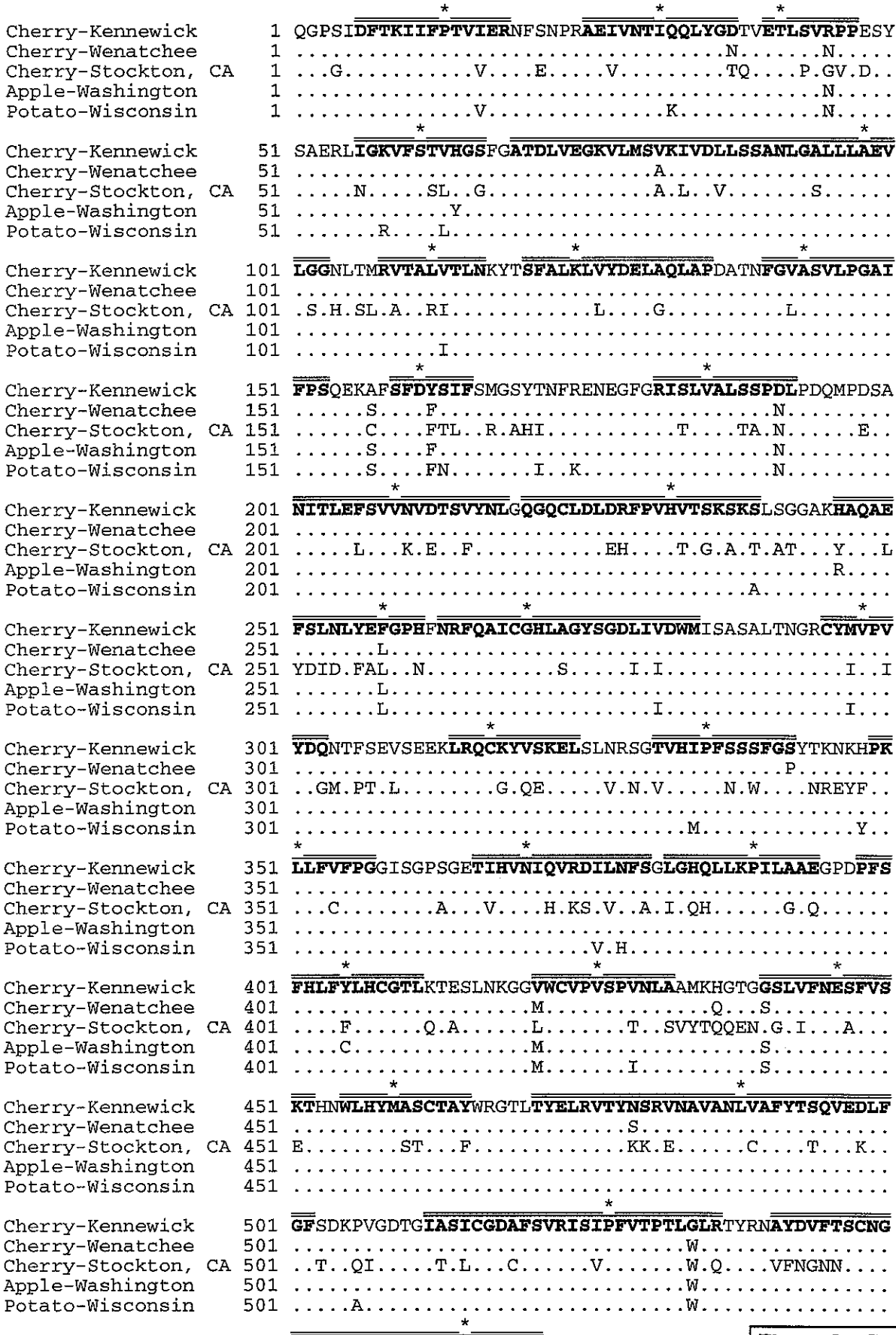

Figure 2. Comparison of the coat proteins encoded by CRLV. A dot indicates identity. A line above a sequence & bold type indicates potential antigenic sites, and the asterisk denotes the most antigenic residue.